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# Does genetic variation and gene flow vary with rarity in obligate seeding *Persoonia* species (Proteaceae)?

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**Abstract**

Theory predicts that genetic variation is a determinant of persistence, and that the abundance and distribution of variation is strongly dependent on genetic drift and gene flow. Small, isolated populations are expected to be less diverse and more differentiated than large, inter-connected populations. Thus rare species may be more at risk of extinction. We used 389 putative AFLP loci to compare genetic variation and structuring in two pairs of closely-related common (large populations geographically widespread) and rare (small populations spatially restricted) *Persoonia* species. We genotyped 15–22 adult plants, from four populations, covering the geographic range of each species. Although genetic diversity was low for all four species (for long-lived outcrossing perennials), we found significantly more diversity within populations of the rare species than within those of the common species. AMOVA revealed significant levels of structure both among species (21%) and populations (15%). The proportion of inter-population variation within species did not vary consistently with rarity (Pair 1 rare 21.1% versus common 16.5%; Pair 2 rare 15.8% versus common 20.6%). However populations of the rare species were more differentiated than common species with similar geographic separation, suggesting greater gene flow between populations of the common species. Therefore, even relatively small genetically isolated populations of rare *Persoonia* species were more diverse than large populations of common *Persoonia* species. We hypothesise that common *Persoonia* species have undergone a rapid range expansion from a narrow gene pool, while genetic diversity is maintained in the soil seed-bank of rare remnants.

## Introduction

Genetic variation is considered to be a key predictor of the persistence of population's as it determines a population's capacity for adaptive change (Saccheri et al. 1998). Theory predicts that small isolated populations will lose genetic variation and diverge genetically as a result of random drift and limited gene flow (Wright 1931, 1951). Therefore, populations of rare species are expected to have low levels of variation and limited ability to adapt to changing environmental conditions. However, despite recent efforts to verify these features of populations few generalities seem to be emerging about the relationship between plant rarity and genetic variation (Gitzendanner and Soltis 2000; Nybom 2004; but see Cole 2003). This may reflect the nature of the comparisons that have been made, as results are likely to differ depending on the criteria used to determine rarity (based on local abundance, geographic range, and habitat specificity; Rabinowitz 1981) and the life history attributes of the species. Ideally we should compare closely related species groups or pairs with similar history, breeding systems, pollinators and dispersal strategies, which differ in their level of rarity.

Here we compare the level of genetic variation and structuring between common and rare perennial shrubs in the genus *Persoonia* (Proteaceae). The Proteaceae are largely a Southern Hemisphere family with Australia (46 genera and almost 1100 species) and southern Africa (14 genera and c. 387 species) as its centres of greatest diversity. *Persoonia* is a genus of 100 species, all endemic to Australia (Weston 1995). We selected two pairs of closely related taxa (hereafter referred to as species), with sharply contrasting local abundances and geographic distributions. One species pair is *Persoonia mollis* subspecies (ssp.) *nectens* S.L. Krauss & L.A.S. Johnson (common) and *P. mollis* ssp. *maxima* S.L. Krauss & L.A.S. Johnson (rare). The other pair (previously considered to be part of the same species complex) is *P. lanceolata* Andrews (common) and *P. glaucescens* Sieber ex Spreng (rare). Based on morphological and genetic characters (Weston 1995, 2003), the species in each pair are more closely related to each other than to other species in the genus. We define rarity based on local abundance and geographic distribution i.e. rare species have $< 1000$ plants within any known population and an area of occurrence $< 500$ km$^2$ (based on IUCN criteria for endangered species).

The four *Persoonia* species are all erect shrubs occurring in south-eastern Australia (Weston 1995), and their current distribution and abundance probably reflect the effects of climate change, fire and habitat fragmentation. In this instance, there is no evidence to suggest that the distributions of the rare species have changed substantially in recent times (based on New South Wales herbarium specimen data for the past 110 years; Rymer et al. unpublished). Populations of the common and rare species pairs are affected by fragmentation of the landscape to similar degrees, but the two pairs of species have been differently affected by natural and anthropogenic disturbances. Both *P. mollis* subspecies have patchy distributions within relatively continuous bushland, while recent urban and agricultural development (predominantly within the last 150 years) has subdivided populations of both *P. lanceolata* and *P. glaucescens*. Our study compares species with similar life-history traits (habitats, plant structure and gross fruit and flower morphology) and phylogenies (Weston 1995; Krauss 1998). These species are obligate outcrossers (Rymer et al. 2005) sharing the same suite of native bee pollinators (Bernhardt and Weston 1996; Wallace et al. 2002). All *Persoonia* species produce fleshy fruits capable of being dispersed by large mammals and birds (Benson and McDougall 2000) although most seeds are expected to develop within close proximity to parent plants. These fire sensitive species rely on seed stored in the soil to re-establish after fire, which kills standing plants. Fire triggers seed germination, but plants are slow to mature and replenish the seed-bank (6–7 years to flower; Myerscough et al. 2000) leaving populations at risk of extinction from repeated fire events in close succession.

Here we predict that the effect of sustained rarity on population genetic attributes will be that the rare species will have: (1) less total intraspecific genetic variation than common species with greater geographic ranges that are exposed to a wider range of environmental conditions; (2) less genetic variation within populations than common species, reflecting the increased susceptibility of small populations to lose variation through genetic drift; and (3) greater genetic differentiation among populations as a consequence of lower gene flow.

## Methods

### *Site description*

For each of the four *Persoonia* species, we sampled four populations distributed across the geographic range of each species (Figure 1). This means that geographic distances between populations is greater for common than for rare species. We measured for each population the area covered, and the number of adult plants present (Table 1). Once the population boundaries were determined, we randomly selected 15–22 adults plants along a haphazard walk covering the whole population. Leaf and floral buds collected from each plant were frozen immediately in liquid $N_2$ and then stored at $-80$ °C prior to DNA extraction.

### *AFLP procedure*

AFLP analysis followed the method of Vos et al. (1995). Total cellular DNA was extracted from frozen leaf material using a CTAB procedure described by Doyle and Doyle (1987), modified by the addition of Protinase K during the initial

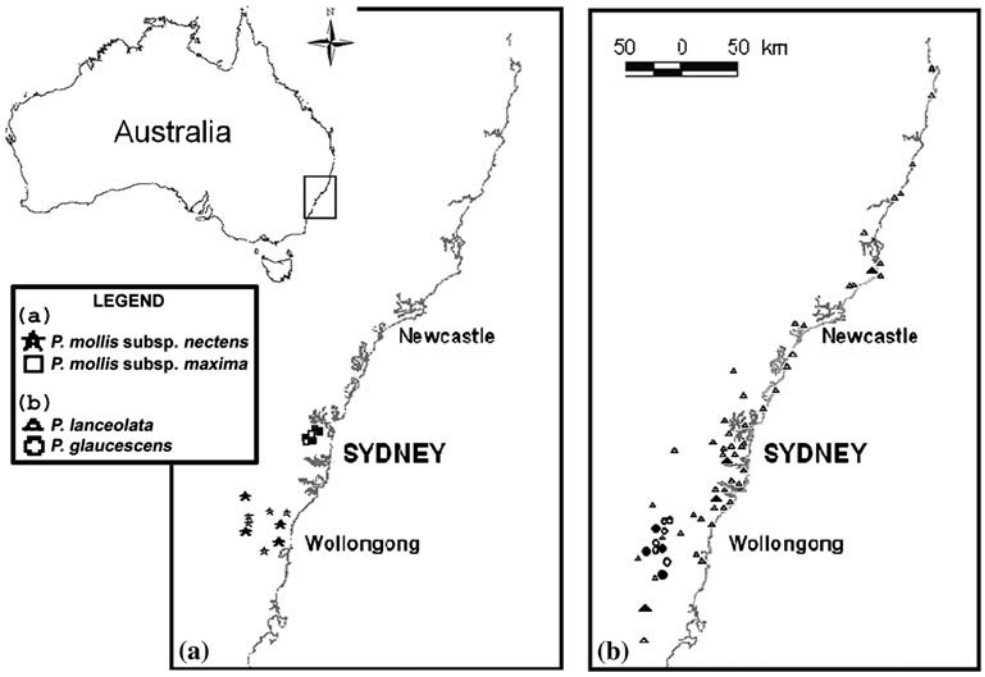


*Figure 1.* Map of the greater Sydney district (Australia) showing the geographic distribution of *P. mollis* ssp. *nectens*, *P. mollis* ssp. *maxima*, *P. lanceolata* and *P. glaucescens*. The records were compiled from the New South Wales National Herbarium. Populations sampled for genetic analysis are filled symbols.

incubation (65 °C/60 min) and RNAse during DNA suspension (37 °C/30 min). To test for reproducibility, random duplicate DNA samples were extracted. Restriction digestion of genomic DNA was carried out in a total volume of 25$\mu$l containing 1.25 U *Eco*RI/*Mse*I, 125 ng of DNA, 12$\mu$l adapter solution, 0.5 U T4 DNA Ligase, buffer and DNA-free water using AFLP$^{TM}$ Core Reagent Kit (Life Technologies), then diluted 1:10 in TE buffer. Pre-selective PCR was performed in a 25$\mu$l total volume containing 2.5$\mu$l 10× PCR buffer, 1.5 mM $MgCl_2$, 20$\mu$l Preamp Primer Mix (Life Technologies), 1 U *Taq* DNA Polymerase (Perkin Elmer), 2.5 ng restricted DNA template and DNA-free water. PCR was performed with a Perkin-Elmer Applied Biosystems 9700 thermal cycler with heated lid programmed for 20 cycles each at 94 °C for 30 s, 56 °C for 2 min, 72 °C for 2 min. A final extension step at 60 °C for 30 min was performed after 20 cycles. PCR products were diluted 1:20 with TE buffer for subsequent selective amplification. For selective amplification three primer pairs were selected. Selective PCR was carried out in a 20$\mu$l total volume containing three *Eco*RI fluorescent primers including: 7 ng of E-aca (fam), 7 ng of E-agg (hex), and 7 ng E-acc (ned); 15 ng of M-cacg primer, 0.2 mM of each of four dNTP, 0.25 U *Taq* DNA Polymerase (Perkin-Elmer), 2.5$\mu$l of diluted preselective PCR product, 1.5 mM $MgCl_2$, 2.0$\mu$l 10× PCR buffer and DNA-free water. A touch-down PCR was initiated with 1 cycle at 94 °C for 2 min, 70 °C for 1 min, 72 °C for 2 min followed by eight cycles starting at 94 °C for 30 s, 69 °C for 1 min, 72 °C for 2 min reducing annealing temperature by a 1.0 °C after each cycle, followed by 23 cycles at 94 °C for 30 s, 61 °C for 1 min, 72 °C for 2 min. A final extension step at 60 °C for 30 min was performed after 23 cycles. To test reproducibility, duplicate reactions were run. Following selective PCR the fluorescently labelled fragments were visualised on 5% polyacrylamide gels with an ABI Prism 377XL sequencer (Applied Biosystems). Four multiplexed dyes constituted a fingerprint for each individual. Three dyes represented primers used in the PCR and fourth dye included internal size standard. The fingerprints were visualised using ABI Genescan software. AFLP profiles were scored for presence or absence of fragments, based on minimum fragment intensity. Initial trials indicated

*Table 1*. The location, estimated population area, number of adult plants and voucher number for the study sites selected for genotyping to compare the two pairs of common and rare *Persoonia* species.

| *Species* (species pair, plant rarity) | | | | |
|---|---|---|---|---|
| Site (abbreviation) | Location[a] | Population area[b] | Number of adult plants[c] | Herbarium voucher[d] |
| *P. mollis* ssp. *nectens* (pair 1, common) | | | | |
| Colo Vale (CO) | 34°23′45″ E, 150°27′57″ S | 2.2 | 300 | 714581 |
| Mount Kembla (KE) | 34°26′29″ E, 150°48′11″ S | 1.0 | 300 | 714582 |
| Loddon Falls (LO) | 34°17′12″ E, 150°53′42″ S | 3.1 | 500 | 714583 |
| Nattai (NA) | 34°08′10″ E, 150°29′10″ S | 4.7 | > 1000 | 714584 |
| *P. mollis* ssp. *maxima* (pair 1, rare) | | | | |
| Binya Close (BI) | 33°38′41″ E, 151°06′55″ S | 1.2 | 60 | 714587 |
| Flinders Place (FL) | 33°39′28″ E, 151°06′44″ S | 1.8 | 100 | 714586 |
| Galston Creek (GC) | 33°39′21″ E, 151°04′12″ S | 1.5 | 100 | 714585 |
| Ku-ring-gai (KU) | 33°40′34″ E, 151°8′02″ S | 2.2 | 200 | 714588 |
| *P. lanceolata* (pair 2, common) | | | | |
| Bundeena (BU) | 34°06′11″ E, 151°06′00″ S | 5.3 | > 1000 | 714589 |
| Crowdy Head (CR) | 31°48′49″ E, 152°43′39″ S | 3.3 | > 1000 | 710502 |
| Meryla Road (ME) | 34°38′21″ E, 150°24′11″ S | 2.0 | 300 | 710519 |
| Ryland Track (RY) | 33°42′10″ E, 151°12′18″ S | 2.5 | 800 | 714590 |
| *P. glaucescens* (pair 2, rare) | | | | |
| Berrima (BE) | 34°28′03″ E, 150°20′52″ S | 0.8 | 40 | 710520 |
| Joadja (JO) | 34°23′47″ E, 150°19′04″ S | 3.3 | 300 | 710513 |
| Robertson (RO) | 34°31′28″ E, 150°34′09″ S | 3.8 | 300 | 714591 |
| Welby (WE) | 34°26′00″ E, 150°24′55″ S | 2.1 | 200 | 714592 |

[a]Based on GPS position using datum WGS1984.
[b]Estimated area in hectares based on field survey.
[c]Estimated number of flowering individuals per population.
[d]Vouchers lodged at the New South Wales National Herbarium.

that fragments between 100 and 400 bp in size were highly repeatable, and therefore these were used for further analysis.

### *Genetic analysis*

We conducted this analysis in two ways, designed to compare the level of genetic variation within populations and species for all four study species. Firstly using the fragments produced by the species, and secondly using only the fragments common to all species.

We estimated the allele frequencies at each locus using a Bayesian method assuming non-uniform prior distribution of allele frequencies (Zhivotovsky 1999) using the computer program AFLP-surv 1.0 (Vekemans et al. 2002). Estimates of allele frequencies were used to calculate the unbiased expected heterozygosity (*He*) (Lynch and Milligan 1994) (AFLP-surv 1.0), percentage of polymorphic loci (*P*) (calculated in Popgen 1.32), and the Shannon information index (Shannon's *I*) (Popgen 1.32) (Shannon and Weaver 1949).

At the species level, we tested for significant differences between common and rare species in the levels of genetic variation with a one-way analysis of variance (ANOVA, using SAS v8). The raw data were *P*, *He*, and Shannon's *I* based either on the fragments found in each species or on the fragments common to all species. Rarity (common or rare) was a fixed factor, with the two species pairs used as replicates. To test for significant differences between the levels of genetic variation within populations of common and rare species we used an ANOVA (using SAS v8), in which species pair (Pair) and plant rarity (Rarity) were fixed factors in the analysis. Each Pair by Rarity combination had four replicate populations.

We used an analysis of molecular variance (AMOVA) to assess the amount of genetic variation

partitioned among species and populations and to determine the level of genetic structure within species (Weir and Cockerham 1984; Excoffier et al. 1992; Weir 1996). The program ARLEQUIN (Schneider et al. 1997) computed the variance components among species, among populations, and within populations. The significance of the components was tested using a hierarchical, non-parametric permutation approach (Excoffier et al. 1992). To calculate the level of genetic structuring within each of the four species we used an AMOVA within the computer program GenAlEx v6 (Peakall and Smouse 2004). This program computes the partitioning of genetic variation within and among populations, as well as Wright's $F_{ST}$ within the species to assess genetic differentiation among populations (assuming that each locus is a two allele system, the markers are selectively neutral, and in gametic-phase equilibrium; Lynch and Milligan 1994). The significance of $F_{ST}$ was tested by comparing the observed $F_{ST}$ with a null distribution created by 1000 random permutations of individuals among populations.

To test whether the genetic differentiation among populations within the species was caused by geographic isolation (isolation by distance model), we used a MANTEL test (Mantel 1967). This test performed in ARLEQUIN (Schneider et al. 1997) compares the association between pairwise $F_{ST}/(1-F_{ST})$ (using ARLEQUIN) and log geographical distances among populations (Rousset 1997).

## Results

The three AFLP primer pairs produced a total of 389 fragments (putative loci) scored between 100 and 400 bp from the four *Persoonia* species (339 samples), none of which were present in all samples across species. While the high density of fragments suggests that there may be some size homplasy, the consistency of fragment peak heights and shapes (well characterised by Krauss 1999, 2000) suggests that this is not a major problem. Size homplasy has the potential to reduce estimates of expected heterozygosity but this is expected to occur at similar levels in all species, and therefore would not alter the comparisons made between common and rare species. All three primers produced a large number of fragments (M-cacg E-aca produced 136, E-agg 129, and E-acc 124 fragments). The number of fragments amplifying in at least one individual varied among the species pairs (*P. mollis* ssp. *nectens* and ssp. *maxima* produced 282 fragments; *P. lanceolata* and *P. glaucescens* produced 335 fragments) and species (*P. mollis* ssp. *nectens* 241 fragments; *P. mollis* ssp. *maxima* 167 fragments; *P. lanceolata* 243 fragments; *P. glaucescens* 266 fragments). The great majority of fragments were polymorphic within species.

We analysed the data for each primer pair separately, and also with all the fragments from the three primer pairs combined. The results of these analyses were similar for each primer pair, so the following results show the analyses conducted on all fragments.

### *Genetic variation*

Species-level genetic variation was significantly different in common and rare species (ANOVA, $df = 1$, $P < 0.05$) based on the fragments that amplified within each species. All estimates of genetic variation calculated (*P*, *He* and Shannon's *I*) were significantly higher in the rare species than in the common species (Table 2). The pattern was similar in both species pairs, although the difference was more pronounced in the *P. mollis* ssp. *nectens*/*P. mollis* ssp. *maxima* pair than the *P. lanceolata*/*P. glaucescens* pair. The rare *P. mollis* ssp. *maxima* had the highest level of *P* (100%), *He* (0.181) and Shannon's *I* (0.290), while the common *P. mollis* ssp. *nectens* had the lowest species-level variation (98.3%, 0.126, 0.213, respectively). Intermediate levels of variation were detected in the *P. lanceolata*/*P. glaucescens* pair, but the common *P. lanceolata* ($P = 98.7\%$, $He = 0.135$, $I = 0.226$) displayed consistently lower levels of variation than its rare congener *P. glaucescens* ($P = 100\%$, $He = 0.154$, $I = 0.255$). The trend was similar when only the fragments common to all species were included in the analysis for *He* and Shannon's *I* (Table 3), but the difference was not significant. The percentage of these common fragments that were polymorphic was not consistently related to rarity.

To estimate the levels of genetic variation within populations, we used the fragments that amplified in each species. An ANOVA designed to compare the levels of genetic variation within

*Table 2.* The level of genetic variation within populations and within species for two pairs of common and rare *Persoonia* species

| Population | n | # loci | *P* | *He* | | Shannon's *I* | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | SE | Mean | SE |
| *P. mollis* ssp. *nectens* (common) | | | | | | | |
| CO | 19 | 241 | 60.2 | 0.123 | 0.009 | 0.187 | 0.048 |
| KE | 20 | 241 | 49.4 | 0.123 | 0.010 | 0.175 | 0.051 |
| LO | 20 | 241 | 51.0 | 0.120 | 0.010 | 0.174 | 0.051 |
| NA | 20 | 241 | 58.5 | 0.131 | 0.010 | 0.193 | 0.050 |
| Population mean | 20 | 241 | 54.5 | 0.124 | 0.010 | 0.182 | 0.050 |
| Species | 79 | 241 | 98.3 | 0.126 | 0.009 | 0.213 | 0.022 |
| *P. mollis* ssp. *maxima* (rare) | | | | | | | |
| BI | 16 | 167 | 60.5 | 0.170 | 0.014 | 0.245 | 0.063 |
| FL | 17 | 167 | 55.1 | 0.173 | 0.014 | 0.243 | 0.064 |
| GA | 18 | 167 | 76.7 | 0.185 | 0.014 | 0.269 | 0.057 |
| KU | 16 | 167 | 52.7 | 0.152 | 0.013 | 0.201 | 0.058 |
| Population mean | 17 | 167 | 61.3 | 0.170 | 0.014 | 0.239 | 0.061 |
| Species | 67 | 167 | 100 | 0.181 | 0.013 | 0.290 | 0.025 |
| *P. lanceolata* (common) | | | | | | | |
| BU | 19 | 243 | 62.5 | 0.137 | 0.010 | 0.202 | 0.051 |
| CR | 18 | 243 | 33.7 | 0.088 | 0.010 | 0.120 | 0.050 |
| ME | 21 | 243 | 53.1 | 0.134 | 0.010 | 0.197 | 0.051 |
| RY | 19 | 243 | 63.4 | 0.154 | 0.011 | 0.226 | 0.055 |
| Population mean | 19 | 243 | 53.2 | 0.128 | 0.010 | 0.186 | 0.052 |
| Species | 77 | 243 | 98.7 | 0.135 | 0.009 | 0.226 | 0.023 |
| *P. glaucescens* (rare) | | | | | | | |
| BE | 21 | 266 | 62.8 | 0.156 | 0.010 | 0.224 | 0.052 |
| JO | 20 | 266 | 62.0 | 0.142 | 0.010 | 0.209 | 0.051 |
| RO | 18 | 266 | 60.5 | 0.144 | 0.009 | 0.219 | 0.054 |
| WE | 19 | 266 | 58.7 | 0.149 | 0.010 | 0.213 | 0.053 |
| Population mean | 20 | 266 | 61.0 | 0.148 | 0.010 | 0.216 | 0.052 |
| Species | 78 | 266 | 100 | 0.154 | 0.009 | 0.255 | 0.024 |

We calculated these measures of genetic variation for each species separately (including only the fragments it produced) in AFLP-surv 1.0 and Popgen 1.31. n = Number of individuals genotyped. # loci = Number of fragments scored for the species. *P* = Percentage of polymorphic fragments of any frequency. *He* = Mean expected heterozygosity averaged over all loci. Shannon's *I* = Shannon's information index. See Table 1 for full population names.

populations (n = 4) of the two pairs of common and rare *Persoonia* species found plant rarity to be a significant factor. The common species had significantly less genetic variation within populations than the rare species (*P*, $F = 12.06$, $P = 0.0046$; *He*, $F = 16.30$, $P = 0.0016$; Shannon's *I*, $F = 10.02$, $P = 0.0081$; Table 2). Both rare species had 10% more polymorphic loci than their closely related common species (61.3% *P. mollis* ssp. *maxima* versus 54.5% *P. mollis* ssp. *nectens*; 61.0% *P. glaucescens* versus 53.2% *P. lanceolata*). The level of *He* and Shannon's *I* we found in the rare *P. glaucescens* ($He = 0.148$, $I = 0.216$) were more than 15% greater than the closely related common *P. lanceolata* ($He = 0.128$, $I = 0.186$), and more than 30% greater in *P. mollis* ssp. *maxima* (rare; $He = 0.170$, $I = 0.239$) than in *P. mollis* ssp. *nectens* (common; $He = 0.124$, $I = 0.182$) (Table 2). When the analysis was repeated with only the fragments common to all species, the common species had lower levels of *He* than their rare congeners in both species pairs (Table 3) but the difference was not significant. The *P* and Shannon's *I* were not consistently related to rarity, and the trend for the less variation in the common species was only found in the *P. lanceolata*/*P. glaucescens* pair (based on common fragments Table 3).

*Table 3*. The level of genetic variation within populations and within species for two pairs of common and rare *Persoonia* species.

| Population | n | #loci | *P* | *He* | | Shannon's *I* | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | SE | Mean | SE |
| *P. mollis* ssp. *nectens* (common) | | | | | | | |
| CO | 19 | 90 | 78.9 | 0.215 | 0.017 | 0.361 | 0.056 |
| KE | 21 | 90 | 62.2 | 0.219 | 0.019 | 0.317 | 0.062 |
| LO | 20 | 90 | 65.6 | 0.212 | 0.020 | 0.311 | 0.059 |
| NA | 20 | 90 | 75.6 | 0.236 | 0.018 | 0.376 | 0.059 |
| Population mean | 20 | 90 | 70.6 | 0.221 | 0.019 | 0.341 | 0.059 |
| Species | 80 | 90 | 96.7 | 0.227 | 0.017 | 0.417 | 0.027 |
| *P. mollis* ssp. *maxima* (rare) | | | | | | | |
| BI | 16 | 90 | 72.2 | 0.233 | 0.020 | 0.359 | 0.067 |
| FL | 17 | 90 | 65.6 | 0.234 | 0.021 | 0.338 | 0.065 |
| GA | 18 | 90 | 81.1 | 0.249 | 0.020 | 0.393 | 0.055 |
| KU | 16 | 90 | 57.8 | 0.187 | 0.019 | 0.274 | 0.065 |
| Population mean | 17 | 90 | 69.2 | 0.226 | 0.020 | 0.341 | 0.063 |
| Species | 67 | 90 | 96.7 | 0.246 | 0.018 | 0.454 | 0.023 |
| *P. lanceolata* (common) | | | | | | | |
| BU | 20 | 90 | 84.4 | 0.248 | 0.018 | 0.412 | 0.054 |
| CR | 18 | 90 | 48.9 | 0.146 | 0.019 | 0.210 | 0.057 |
| ME | 21 | 90 | 66.7 | 0.200 | 0.019 | 0.320 | 0.058 |
| RY | 18 | 90 | 84.4 | 0.261 | 0.019 | 0.395 | 0.054 |
| Population mean | 19 | 90 | 71.1 | 0.214 | 0.019 | 0.334 | 0.056 |
| Species | 79 | 90 | 100 | 0.226 | 0.018 | 0.422 | 0.024 |
| *P. glaucescens* (rare) | | | | | | | |
| BE | 21 | 90 | 78.9 | 0.247 | 0.017 | 0.374 | 0.054 |
| JO | 20 | 90 | 78.9 | 0.218 | 0.018 | 0.361 | 0.054 |
| RO | 17 | 90 | 73.3 | 0.209 | 0.017 | 0.372 | 0.065 |
| WE | 19 | 90 | 70.0 | 0.211 | 0.017 | 0.347 | 0.061 |
| Population mean | 19 | 90 | 75.3 | 0.221 | 0.017 | 0.363 | 0.059 |
| Species | 77 | 90 | 97.8 | 0.233 | 0.017 | 0.433 | 0.024 |

We calculated these measures of genetic variation using only the 90 fragments that were common to all species in AFLP-surv 1.0 and Popgen 1.31. n = Number of individuals genotyped. # loci = Number of fragments scored for the species. *P* = Percentage of polymorphic fragments of any frequency. *He* = Mean expected heterozygosity averaged over all loci. Shannon's *I* = Shannon's information index. See Table 1 for full population names.

### *Genetic structure*

Analysis of Molecular Variance (AMOVA) revealed that there were significant levels of variation among species ($P < 0.001$) and populations ($P < 0.001$). In our analysis of the total data set 20.5% of variation was attributed to differences among species, and 14.9% to population differentiation. When analysing the species pairs separately, we found similar levels of genetic variation partitioned among populations for both species pairs (*P. mollis* ssp. *nectens*/*P. mollis* ssp. *maxima* 16.1%; *P. lanceolata*/*P. glaucescens* 15.7%). The proportion of inter-population variation did not vary consistently with rarity when we analysed the data separately for each species. We found relatively more variation among populations for the rare *P. mollis* ssp. *maxima* ($Ø = 21.1\%$, $P = 0.001$) than for closely related common *P. mollis* ssp. *nectens* ($Ø = 16.5\%$, $P = 0.001$). However, we also found that populations of *P. glaucescens* (rare $Ø = 15.8\%$, $P = 0.001$) were less differentiated than *P. lanceolata* populations (common $Ø = 20.6\%$, $P = 0.001$).

The relationship between genetic and geographic distance was more pronounced among populations for the common species (*P. mollis* ssp. *nectens* $r^2 = 0.67$; *P. lanceolata* $r^2 = 0.90$) than for

the rare species (*P. mollis* ssp. *maxima* $r^2 = 0.23$; *P. glaucescens* $r^2 = 0.11$). Of these *Persoonia* species, we only detected a significant correlation between genetic and geographic distance in the common *P. lanceolata* (MANTEL; $P = 0.035$), which has the greatest geographic range (> 380 km). The closely related rare *P. glaucescens* has a much smaller geographical range (< 30 km), however the genetic distance between populations is similar to its common relative (*P. lanceolata* $F_{ST} = 0.071$ to 0.320, *P. glaucescens* $F_{ST} = 0.090$–0.229) (Figure 2).

## Discussion

### *Does genetic variation relate to plant rarity?*

Empirical studies exploring the relationship between genetic variation and plant rarity have produced mixed results, depending on the type of comparison employed. Different types of rarity, measured in terms of local abundance, geographic range and habitat specificity (Rabinowitz 1981), as well as the duration of rarity, may have very different genetic implications. In our study, we

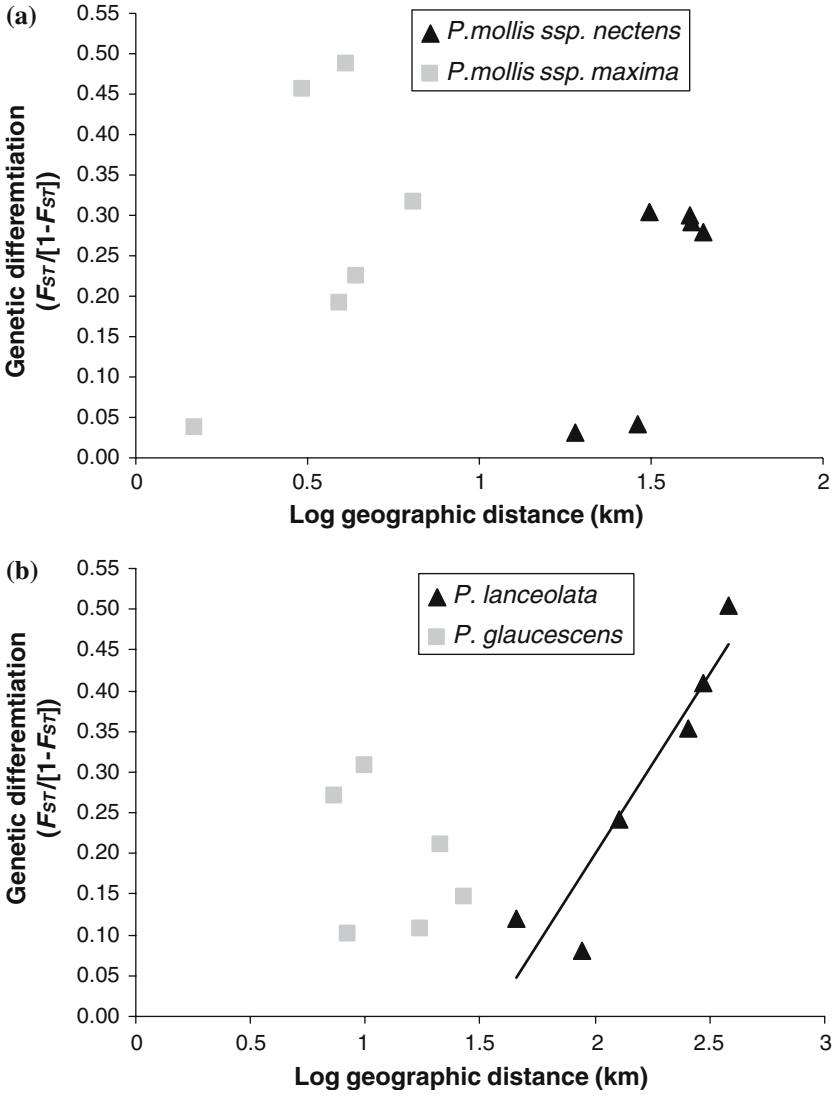


*Figure 2.* The relationship between genetic differentiation and genetic distance among populations of the common and rare *Persoonia* species. (a) The common *P. mollis* sp. *nectens* and and the closely related rare *P. mollis* sp. *maxima*. (b) The common *P. lanceolata* and the closely related rare *P. glaucescens*. Common species are represented by black triangles and rare species by grey squares. The black line represents the 'line of best fit' ($y = 0.443 \times -0.686$) for *P. lanceolata* (MANTEL $r^2 = 0.896$ $P = 0.035$).

compare closely related species pairs that differ dramatically in terms of their local abundance and geographic range (Table 1) (with similar habitat requirements), both of which are likely to be consequences of climate change since the late Pleistocene (Pickett et al. 2004).

We found a consistent relationship between total intra-species genetic variation and rarity. In our analysis of variance testing for the effect of plant rarity both pairs of closely related species showed the same response, having greater levels of genetic variation in the rare species than in the common species. The rare *P. mollis* ssp. *maxima*, which has the most restricted geographic distribution has the most within species genetic variation. Despite the fact that *P. glaucescens* covers a geographic range less than one fourteenth the area encompassing *P. lanceolata*, and that it is restricted to open woodlands in montane locations (unlike *P. lanceolata* that is found in coastal heath and montane woods), it appears that the greater geographic range and wider habitat preference found in the common *P. lanceolata* is not associated with greater genetic variation. This goes counter to the idea that common species have more genetic variation than rare species (Frankham et al. 2002). A potential explanation could be that local selection and drift in several small isolated populations of *P. glaucescens* has increased genetic variation across the species (Altukhov 1981).

The amount of genetic variation within populations (intra-population variation) is an important determinant of the ability for populations to persist (Saccheri et al. 1998), particularly in unpredictable environments (e.g. fire prone habitats). It is well-established in theory that small populations will lose genetic variation over time as a result of genetic drift (Wright 1931). Rare species generally have lower levels of genetic variation within populations than related common species (Loveless and Hamrick 1984; Cole 2003; Nybom 2004). However, numerous exceptions to this general trend have been found. For example, in a meta-analysis, Cole (2003) found 10 out of 48 comparisons in which rare species have higher levels of polymorphism than common species and 14 out of 54 comparisons in which rare species have higher levels of heterozygosity. The rare species we studied, which had relatively small standing populations, had similar (or even greater) levels of genetic variation than closely related common species with much larger populations. We therefore reject our prediction that "the rare *Persoonia* species will have less genetic variation within populations than common *Persoonia* species, reflecting the increased susceptibility of small populations to lose variation through genetic drift". One possible explanation is that our estimate of population size is likely to underestimate the effective population size of these *Persoonia* species, all of which can develop substantial seed-banks in the soil (Auld et al. 2000). A persistent seed-bank provides the opportunity for gene flow over time (between the offspring of multiple cohorts) and can potentially buffer populations against the effects of genetic drift (Llorens et al. 2004). Furthermore, there may not have been sufficient time to detect the recent effects of habitat fragmentation ($< 150$ year ago) in these long-lived perennials (Rossetto et al. 1995). The differences we detected in the genetic variation between common and rare species (Table 2) appear to be counter-intuitive (and in the opposite direction to that predicted based on population size differences). This relationship is intriguing and warrants further investigation to identify the underlying mechanisms; does it reflect rapid colonization in the common species?

Overall, the average levels of genetic variation within populations of all four *Persoonia* species were low ($He < 0.17$; Table 2) for long-lived perennial species ($He = 0.25$) with an outcrossing breeding system ($He = 0.27$) and seed dispersal through animal ingestion ($He = 0.24$) based on dominant markers (Nybom 2004). This may be due to historical bottlenecks, potentially attributed to continental drying during glaciation events (Pickett et al. 2004). The predicted relationship between genetic variation and drift assumes that the system is at equilibrium (Wright 1969). In reality while the general distribution of these species may have been relatively constant for thousands of years, metapopulation processes may reduce genetic variation (Pannell and Charlesworth 2000) in these fire prone landscapes through continual colonization and extinction events in these obligate-seeding *Persoonia* species. Whatever the mechanism, the question of how this variation relates to population persistence still remains. This requires an understanding of the demographic effect of frequent fire events on population persistence, and potential long-distance seed dispersal

events on population colonization ability, in conjunction with estimates of genetic variation.

*Does limited gene flow cause rarity?*

A plausible explanation for the differences in geographic range between common and rare *Persoonia* species is a more rapid expansion by common species from glacial refugia (Krauss 1998). Assuming that common species are better colonists (and they have been found to have more seed dispersed; Rymer, 2006) they might be expected to have increased levels of gene flow between populations. However, our results suggest that the level of genetic divergence in *Persoonia* species is not consistently related to rarity across their geographic range. A lack of relationship between population differentiation ($F_{ST}$) and geographic range has also been found in recent reviews (Cole 2003; Nybom 2004). Plant breeding systems and seed dispersal mechanisms have been found to be more important in determining population differentiation (Cole 2003; Nybom 2004). As closely related species typically share similar breeding systems and dispersal mechanisms, genetic distances between populations may not be expected to differ in these comparisons despite the sharply contrasting geographic ranges.

To compare population genetic differentiation we should standardise the separation of populations. Spatial analysis (such as MANTEL's test) allows for comparison of the genetic differentiation between populations of species that differ in geographic range by relating genetic and geographic distance (Heywood 1991). We found genetic differentiation to be significantly correlated with geographic distance for populations of the common *P. lanceolata* (explaining 89.6% of the variation, $P = 0.035$; Figure 2). No relationship was found for the other species, which had considerably smaller geographic ranges, suggesting that isolation by distance model does not apply at these smaller spatial scales. Nevertheless, this enables us to compare the level of genetic differentiation found in the rare *P. glaucescens* to its common congener. The maximum genetic distance found in the rare species ($F_{ST} = 0.229$) with a geographic range of only 30 km equates to up to 10 times the geographic distance in the common *P. lanceolata* (approximately 300 km) (Figure 2). The other species pair shows a similar pattern, with the common *P. mollis* ssp. *nectens* having less genetic differentiation ($F_{ST} = 0.223$ versus 0.318) despite having a larger geographic range than its rare congener (*P. mollis* ssp. *maxima*; approximately 44.6 km versus 6.5 km). Assuming genetic differentiation is inversely related to gene flow (Wright 1951), this indicates that common *Persoonia* species have greater levels of gene flow between populations, supporting the findings of the meta-analysis performed by Cole (2003) (based on $F_{ST}$ in 65% of the 37 comparisons). Further research investigating the levels of gene flow in common and rare species at similar spatial scales using both indirect and direct methods needs to be done to confirm this relationship.

*Conclusion*

This study provides an important first step in understanding the association between genetic variation and plant rarity in fire sensitive *Persoonia* species. Our finding that the rare species contained greater genetic variation than the common species goes against expectations based on theory. However given the potential for soil seed-banks to buffer populations against the effects of genetic drift and the relatively recent effect of habitat fragmentation, these species may require more time for effects of population size to be detected. We propose that the most likely explanation for the lower genetic variation found in common *Persoonia* species is rapid range expansion through long-distance dispersal events from a narrow gene pool. Our findings that common species have higher levels of gene flow support this hypothesis, but further investigations are required to determine historical colonization rates for common and rare species. This study will provide a valuable base line for future investigations into the long-term effects of anthropogenic disturbances in this group.

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